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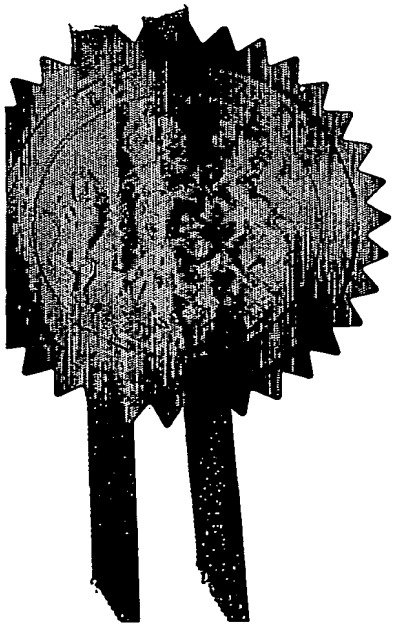

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Request for grant of a patent

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Cardiff Road
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. Your reference — P23064/PKE/BOU

:. Patent application number *(The Patent Office will fill in this part)* — 9920974.4 | -7 SEP 1999

:. Full name, address and postcode of the or of each applicant *(underline all surnames)* — Giltech Limited
9/12 North Harbour Estate
AYR
KA8 8AA

Patents ADP number *(if you know it)* — 40/5822002 TS

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — "Growth Substrate"

5. Name of your agent *(if you have one)* — Murgitroyd & Company

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — 373 Scotland Street
GLASGOW
G5 8QA

Patents ADP number *(if you know it)* — 1198013

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
–		

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*
–	

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

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Continuation sheets of this form	
Description	1
Claim(s)	–
Abstract	–
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Priority documents	–
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	–
Request for preliminary examination and search *(Patents Form 9/77)*	–
Request for substantive examination *(Patents Form 10/77)*	–
Any other documents *(please specify)*	–

11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Co Date 6.9.1999

Murgitroyd & Company

12. Name and daytime telephone number of person to contact in the United Kingdom Beverley Ouzman 0141 307 8400

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"GROWTH SUBSTRATE"

S.H.Cartmell, P.J.Doherty, C.C.M.Thompson*, D.Healy*, T.Gilchrist*
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*Giltech Ltd., 8-12 North Harbour Estate, Ayr KA8 8AA U.K.

INTRODUCTION

Controlled Release Glass (CRG) is a phosphate based material which degrades at a predeterminable rate. The potential for using CRG as a cartilage engineering matrix has been assessed using isolated equine chondrocytes with *in-vitro* techniques. The glass was provided in fibrous form in three different compositions by Giltech Ltd.. The three CRG compositions provided have showed potential as a tissue engineering substrate.

MATERIALS AND METHOD

A total of 200,000 chondrocytes isolated from horse articular cartilage were added to each 2cm well in a 24 well plate. Every well contained 0.02grams of glass fibre sample. Four different fibres (diameters 20-30μm) were analysed: F1 - containing Fe_2O_3 and NaF, F2 - containing Ce_2O_3 and F3 - containing Ce_2O_3 and Se. The culture medium (containing 10% FCS) was changed daily. At time periods of 3 days, 1 week and 2 weeks, the samples were stained using rhodamine phalloidin and oregan green for the viewing of actin and tubulin using a laser scanning confocal microscope. At the same time periods, the cell supernatant was removed and stored at -80°C until analysis on cell viability and type II collagen production could be performed. Production of type II collagen was analysed by using RT-PCR analysis on the cDNA from the chondrocyte population in contact with the glass fibres. The total RNA was prepared from the cell population by the addition of 1ml of TRIzol (SIGMA) to the cell population for 5 minutes. After this time, The TRIzol was retrieved and stored at -80°C until RT-PCR analysis could be carried out. The RT-PCR analysis was performed by tagging with primers for collagen type II and with gapDH for cell viability.
Zymography was also performed at time periods of 4 days, 1 week and 2 weeks for detection of matrix metalloproteinases (MMP's) produced by the chondrocytes.

RESULTS AND CONCLUSIONS

Chondrocytes adhered to all three types of fibre sample. At the 3 day time period, the cells appeared to be rounded. At 1 week and 2 weeks, confocal microscopy indicated cell proliferation between all time periods. At 1 week and 2 weeks, the cells were elongated and formed a monolayer along the fibre length as can be seen in Figure 1.
The RT-PCR analysis showed that fibres F2 and F3 were producing collagen type II up to and including the two week time period indicating that the cells retained their chondrocytic phenotype.
The zymography performed on F2 and F3, showed that the cells in contact with these fibres produced MMP2 at all three time periods, but in a greater quantity at 2 weeks than 1 week, and at 1 week than 4 days. This increase of MMP2 production is expected, as the cells were seen to have increased in number at these time periods from the confocal microscope analysis.
In conclusion, all three fibres types showed cell adherence and the chondrocytes adhered to F2 and F3 appear to retain the ability to produce type II collagen.

Figure 1. Adhered chondrocytes to fibre F1 at 2 weeks.

The authours wish to acknowledge the EPSRC for funding of this work.

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